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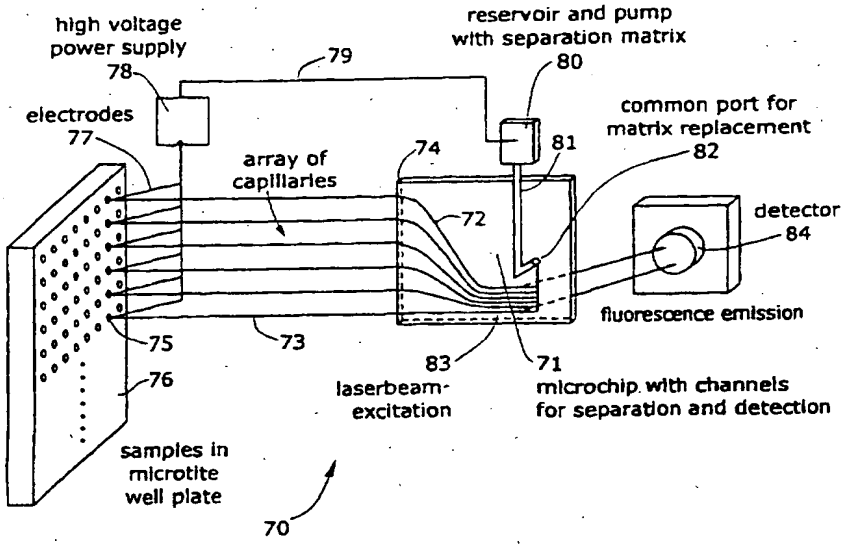

(57) Abstract

A hybrid microfabricated substrate capillary array assembly, which provides an interface between the integrated channels (12) on a microfabricated substrate (10), e.g., microchip, and flexible capillaries (18), is disclosed. The hybrid device permits, e.g., convenient injection of samples from a capillary array (18) into channels (12) in a microchip (10) and also enables, e.g., convenient detection on the device by LIF (laser induced fluorescence). With the use of such an assembly, large numbers of samples may be processed simultaneously, leading to high-speed, high-throughput analyses.

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MICROFABRICATED HYBRID CAPILLARY ARRAY AND MULTICHANNEL DETECTION ASSEMBLY

FIELD OF THE INVENTION

The present invention relates in general to chemical and biological analytical systems.

BACKGROUND OF THE INVENTION

The increasing importance of analytical methods to current chemical and molecular biological research has led to a demand for rapid information turnover from such systems for large numbers of samples. For example, research needs associated with DNA sequencing for the Human Genome Project or with drug screening of natural samples for new therapeutic molecules have generated a great deal of interest in developing high-speed, high-throughput separation and detection methods. One well-known technique that meets these requirements is capillary array electrophoresis, which is frequently used in conjunction with laser-induced fluorescence (LIF) scanning detection. However, LIF scanning techniques have certain drawbacks when conventional detection schemes are employed for analysis if there are many capillaries in an array, of multiple capillaries. For example, one analytical method used if there are many capillaries in an array has detectors or light sources moving at a sufficient speed that peak signals from all of the capillaries will be observed. However, sensitivity may be decreased if this method is used because the integration time for each capillary is limited. Further, the limited data acquisition rate and mechanical vibration noise may also cause problems. Proper alignment of the

capillaries vis-a-vis the coherent light source, so that each capillary receives the intended light intensity, is also very important but is difficult to achieve in practice. Furthermore, multiple capillaries in an array cannot be spaced sufficiently closely to achieve the density required for simultaneous detection of very large numbers of samples.

Separately, techniques that incorporate sophisticated instrumentation on microchips have been developed for analytical chemistry. Multiple miniaturized analytical instruments have been microfabricated on a single chip, enabling techniques that provide high-throughput analysis. Conventional photolithographic technology can be used to fabricate multiple channels on a chip for performing fast parallel assays. However, limited chip space presents a problem when fabrication of many channels of sufficient length to accommodate separation techniques is attempted. In addition, the use of multiple channels requires multiple wells for connection to electrodes, a configuration that is difficult to design and construct.

The limited available surface area on a chip furthermore makes it difficult to introduce sample to the chip by conventional means, particularly in a multi-channel mode. For example, if the chip is configured as a reusable device, sample introduction means are necessary to place a new sample onto the chip for each consecutive analysis. Currently, for example, the chips developed for capillary electrophoresis have large inlet ports for pipetting in the sample. A given sample, once loaded onto the chip, can be analyzed repeatedly; however, different new

samples can be analyzed only with difficulty. Such a system may be a good design for a cheap, disposable device, but it does not allow the chip to be reused easily. Thus, it is desirable to construct a new chip-sample interface for repeatable introduction of different samples into a reusable microfabricated device. This is especially important when the microfabricated device is made of an expensive material, e.g., quartz, or contains complex, difficult to fabricate structures which make the concept of disposability too expensive. Alternatively, such an interface would also be desirable when a sample is to be processed through multiple analytical procedures off chip, e.g., when part of the analysis is performed in a standard non-micromachined instrument, followed by consecutive analytical (e.g., detection) steps on a microfabricated device. An additonal advantage of such a configuration would be that only a portion of an analyzed sample need be applied to a microfabricated device. The remaining portion could be used for other purposes.

SUMMARY OF THE INVENTION

The hybrid microfabricated substrate (e.g., microchip) capillary array assembly of the invention combines the separation power available in capillary electrophoresis with the convenience-of-manipulation capability (e.g., reaction or separation) and detection in the microchip format. In one aspect, the invention is directed to a hybrid microfabricated substrate capillary array assembly that includes one or more

capillaries each having a first cross-sectional shape; a microfabricated substrate including one or more channels for conducting a fluid in the substrate, the one or more channels each having a second cross-sectional shape; and a connecting structure formed in the substrate, connecting the one or more capillaries to the one or more channels in the substrate so as to enable fluid communication between the capillaries and the channels. The capillaries and the channels each may be of any convenient cross-sectional shape, e.g., ellipsoidal, trapezoidal or circular. The channels can also be different shapes in different portions of the substrate. Preferably, the substrate is of a light transmissive material, both the capillaries and the channels are circular in cross-section where they join, and the channels are substantially parallel to one another and lie substantially in the same plane at a position in the substrate where detection might take place. Either the capillaries or the channels can be configured for transport or manipulation (e.g., separation or reaction) of fluid suspended molecular species conducted therethrough. Most preferably, the channels are spaced very closely together at one end and terminate at a common port, which can be configured, e.g., for fluid washing of the channels or for replacement of fluid media, for example separation matrix.

In another aspect, the invention is directed to a multichannel detection assembly that includes a light transmissive microfabricated substrate having a plurality of channels formed therein for conducting a fluid in the substrate,

the channels being positioned substantially parallel to each other and defining a plane in the substrate; a light source having an output directed through the substrate at an angle to the plane, wherein the angle is less than the critical reflective angle of the substrate, the light source output travelling through the plurality of channels; and a detector positioned adjacent to the substrate to receive light transmitted through the channels in the substrate. Preferably, the channels are spaced more closely together at one end than at the other and the light source is positioned so that the light source output travels through the plurality of channels in the plane defined by the channels and at the end of the substrate in which the channels are more closely spaced. The light source and detector may be any combination known to those of skill in the art as likely to detect the molecular species being analyzed. Most preferably, the light source is a laser, the detector is a fluorescence detector and sample analysis is by laser fluorescence detection. Alternatively, the light source could be, e.g., UV light, and the detector could be, e.g., an absorbance detector.

The hybrid microfabricated substrate capillary array assembly of the invention is useful, for example, as an injection system for repeated introduction of different samples into a reusable microfabricated device, e.g. a microchip. Sample separation or other analytical procedures, such as reaction or derivatization, can be carried out directly on the microchip.

In between different sample runs, the channels in the microchip can conveniently be washed via their common termination port. Alternatively, the interface assembly of the invention can be used simply to provide access to an on-chip detection system following various off-chip analytical procedures, e.g., separation of samples in the individual capillaries of the system capillary array. Thus, the individual advantages of the capillary array and microchip environments are successfully combined by the hybrid system of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will be more fully understood from the following detailed description, taken in conjunction with the accompanying drawings, in which:

Fig. 1 is a diagram of an embodiment of a hybrid microchip capillary array assembly of the invention;

Fig. 2A is a diagram of a first embodiment of a chip-to-capillary array interface;

Fig. 2B is a diagram of a second embodiment of a chip-to-capillary array interface;

Fig. 2C is a diagram of a third embodiment of a chip-to-capillary array interface;

Fig. 3A is a diagram of a section of a microfabricated multichannel detection assembly of the invention in which the output of the excitation light source is directed perpendicular to the side edge of the substrate microchip;

Fig. 3B is a diagram of a section of a microfabricated multichannel detection assembly of the invention in which the output of the excitation light source is directed at an angle with respect to the side edge of the substrate microchip;

Figs. 4A and 4B show laser induced fluorescence detection of DNA sequencing reaction products separated on a hybrid microchip capillary array assembly of the invention and a capillary-only instrument, respectively;

Fig. 5 shows an image of the fluorescence signal from all channels of the embodiment of Fig. 1 under side illumination

aligned with a representation of the fluorescence intensity of the image; and

Fig. 6 is a diagram showing a microfabricated hybrid capillary array and multichannel detection assembly of the invention in use for analysis of samples in a microtiter well plate.

DETAILED DESCRIPTION OF THE INVENTION

The microfabricated hybrid capillary array and multichannel detection assembly of the invention includes a number of features which are independently useful. Referring to Fig. 1, in such a hybrid assembly, a microchip 10 is fabricated to contain a plurality of channels 12, useful, e.g., for simultaneous sample detection, each of which is adapted at one end 14 to receive a flexible capillary 16 from an array of capillaries 18. In the

channel arrangement depicted in Fig. 1, channels 12 are spaced further apart in region 13 of the microchip, for ease of coupling with capillary array 18, and are spaced more closely together in region 15 of the microchip to facilitate on-chip detection of separated samples, as will be described in more detail below. Channels 12 terminate by merging to a common port 20. This port provides a place for connection to a common buffer reservoir (e.g., anodic) for all channels and serves as a means to wash or replace any matrix material in the channels.

In the fabrication of a preferred embodiment of the hybrid chip-capillary structure, grooves which are semicircular in cross-section are formed by isotropic etching in mirror image on the surface of two substrates, e.g., glass wafers. The substrates are then bonded together to mate the channel halves and form circular channels in the body of the substrate microchip, to which flexible capillaries, which are similarly circular in cross-section, may be mated. Common photolithographic technology enables fabrication of tightly packed channels on such a chip; for example, in an area as small as 5 cm wide on a glass or fused-silica wafer, it is possible to fabricate 500 channels 50 μm wide, spaced 50 μm apart. The completed assembly, microchip plus attached capillary array, can be supported in a chip holder.

Referring to Fig. 2A, in one embodiment of such a hybrid multichannel capillary array, the diameters 22 of the channels 12 are fabricated so they are the same dimension as the outer

diameter of the capillaries 16 in the array. The capillaries are inserted directly into the chip channels and glued in place. Although this fabrication process is simple, a junction 24 is created in which the size of the resulting passage diameter changes sharply from capillary to channel, which may result in some degradation in resolution of separated samples.

In a second embodiment, a multi-step fabrication process may be used to create channels of different diameters for different segments of the chip. As shown in Fig. 2B, the individual capillaries 16 of the capillary array 18 are inserted into the chip segment with the larger diameter portions 32 of the channels. The capillaries extend no further than the beginning of the chip segment with smaller diameter 34 channel portions (which match the inner diameters of the capillaries). The advantage of this embodiment over a two-chip combination (described below) is that there is no need to align and seal the separately constructed chips.

A further embodiment, as shown in Fig. 2C, comprises two chips 52, 54 of differing channel sizes mated together. On the first chip 54 (which can function as a disposable capillary array holder), channel diameters 56 are the same as, or slightly larger than, the outer diameters of the capillaries in the array. Capillaries 16 are inserted into the channels in the first chip 54, and they are then sealed and blunt cut at the point where they exit the channels. The channels in the second chip 52 are constructed to match the inner diameters 58 of the capillaries.

Since the channels of both chips 52, 54 are measured precisely, it is possible to achieve perfect or near perfect alignment of the capillary array and the channels on the chip. The finished device thus provides a uniform capillary-to-channel inner diameter and the capability for simple replacement of the capillary array. An important aspect of this embodiment is that two chips of different materials can be combined. The first chip 54 can be made of an inexpensive material (such as glass, plastic or polymer) so that it is disposable, since it serves only as a framework or holder for the capillaries. The second chip 52, which can be employed for detection, can be made of a more expensive material, e.g., quartz.

In the hybrid structure of the invention, the flexible capillaries of the array can be used, e.g., for sample injections or for cleaning of the channels on the chip. This structure allows sample injection conduits to be omitted from the chip, leaving more space for channel fabrication. Furthermore, the attached array of capillaries effectively extends the lengths of the channels on the chip. Thus, the capillaries of the array can be used for analyte separation, which eliminates the need for inconvenient and time-consuming preparation of the microchip channels themselves before runs, a shortcoming previously associated with microfabricated chips.

The presented embodiments for introducing samples and supporting fluids to a microchip have the advantage of being easy to fabricate, since no drilling of holes and/or preparation of

wells is required. When the chip is fully machined, the size and position of channels are precise; therefore, the manufacture of the device can be automated. Since microfabrication enables design of complex conduits on a chip, once samples and supporting fluids are introduced, it is possible to conduct most types of chemical operations (reagent addition, separation/mixing, concentration, dilution, etc.) on the chip itself.

The closely spaced channels on the microfabricated chip provide an excellent environment for detection of samples travelling in the channels. For example, detection may be performed by introducing a laser perpendicular to the side edge of the device (i.e., side illumination as shown in Fig. 3A) such that the laser traverses the entire array of channels or by introducing the laser at an angle to the side of the chip. Since the chip is surrounded by air, which has a lower index of refraction than the chip material, the microfabricated device acts as an optical wave guide for the laser light. Multiple reflections occur within the chip to illuminate the solutions in all the channels uniformly.

Laser-induced fluorescence (LIF) detection is often a method of choice for sensitive detection on microfabricated chips. Referring to Fig. 3A, a schematic depiction of an embodiment of an LIF detection system in accordance with the invention is shown. In this embodiment, a laser beam or other excitation light source 60 is introduced at one side, or edge,

62 of the chip 64, parallel to the plane defined by the separation channel(s) 66 and directed through all the channels. It is most convenient to position the laser beam at the region of the chip where the channels are most closely spaced, as depicted in Fig. 1. Emitted fluorescence from molecular species travelling in the channels under the influence of an electric field is detected via a detection system 68, placed adjacent to the body of the microchip on one side or the other of the plane defined by the channels. The refractive index of a buffer solution present in the channels can be adjusted (e.g., by addition of sucrose) to match the refractive index of the device material. The excitation light travels from the first channel to the last, traversing the entire group. In some cases the refractive index of the separation matrix may not match that of the material of the chip, and the excitation laser light may be scattered from the walls between the channels. If such scattering would cause a reduction in the sensitivity of detection, the walls between the channels could be removed at the cross-section at which detection is carried out, to form gaps in the channels, and thus eliminate any laser scatter. Since the gap can be very short (0.1-2 mm), the electric field would not be severely distorted, preventing any channel cross-contamination. Of course, a variety of different illumination schemes for detection known in the field of capillary electrophoresis and micromachined devices can also be used. For example, the laser beam may be shaped into a line to illuminate all the channels from a side of

the chip above or below the plane of the channels, or an array of beams for individual illumination of each channel can be used.

In an alternative detection scheme, as shown in Fig. 3B, the solutions in the individual channels can be illuminated by introducing the laser beam at an angle 68 less than the critical angle of the substrate material, so that inner multireflection occurs. Thus, in this embodiment the device body also functions as a wave guide for the excitation light. The reflected laser beam then illuminates the solution in every channel. Fluorescence detection is again carried out above and/or beneath the device.

These configurations for excitation laser and detector placement provide for less background noise than is common in prior art configurations, and, if the refractive index of the buffer solution matches the chip material, no excitation light will leave the chip body. In a multi-channel detection assembly incorporating the present invention, light absorbed by the buffer solution is very small compared to the total light intensity of the laser; therefore, the laser energy is more efficiently used. Additionally, since all the space on either side of the chip plane defined by the channels is free from interference from the light source, mounting the detector becomes relatively easier.

The following examples are presented to illustrate the advantages of the present invention and to assist one of ordinary skill in making and using the same. These examples are not

intended in any way otherwise to limit the scope of the disclosure.

Example I

Capillary electrophoresis of a single terminator DNA sequencing reaction, which produces fragments having single stranded DNA base lengths ranging from 77 to 503, was carried out in a capillary of 30 cm effective length using 2% w/v liquid polyacrylamide (Fig. 4B) and on a hybrid chip of the invention having a capillary of the same length (Fig. 4A). As can be seen, the time based peak widths do not vary significantly between runs of the different capillary configurations, and therefore, no significant resolution degradation was found for DNA sequencing when a capillary and a channel on a chip were joined (Fig. 4A) compared to the single capillary setup (Fig. 4B).

Example II

The side illumination detection system of the invention was also tested. Referring to Fig. 5, a fluorescent solution of 2×10^{-7} M fluorescein was injected into channels of a microfabricated chip. An excitation laser beam was introduced from the edge of the chip, parallel to the plane of the channels in the chip. As the beam was reflected within the chip, it illuminated all the channels. The upper panel of Fig. 5 shows the fluorescence signals from all channels of the chip, which were recorded by a CCD camera. The lower panel of Fig. 5 was

generated from the upper panel data, by measuring the intensity profiles of the fluorescent spots, and shows that the fluorescence intensity of each channel is relatively uniform. This example illustrates that multiple channels can be illuminated from the side of the chip with relatively even fluorescence emission.

Example III

Fig. 6 is a simplified depiction of a multichannel detection assembly 70 in accordance with the invention in use to screen samples from a microtiter well plate. Detection assembly 70 features a microfabricated chip 71 containing a plurality of channels 72 of a circular cross-section, which have been filled with a separation matrix. The channels 72 are mated to external capillaries 73 via connecting structures 74, constructed substantially as described above, and the capillaries are glued in place. The array of external capillaries 73 serves as an injection apparatus to transfer samples to microchip 71 for analysis, in the following manner.

The ends of capillaries 73, distant from the microchip, are inserted in open wells 75 of microtiter plate 76, which contain the samples to be analyzed. Electrodes 77, which are also inserted in microtiter plate wells 75, are electrically connected to high voltage power supply 78. Power supply 78 provides the electromotive force necessary to move sample analytes and fluid through the capillaries 73 to the channels of microchip 71. High

voltage power supply 78 is further connected via power line 79 to buffer reservoir and pump 80, which is in fluid communication with microchip channels 72 via conduit 81 inserted in common channel termination port 82, to complete the electrical circuit.

Upon the application of an electromotive force from power supply 78, charged analytes from sample wells 75 are transferred via capillaries 73 to channels 72 in the microchip, where they are separated in the separation matrix contained in the channels. Laser 83, which is aligned to direct the laser output across all of channels 72, is used to excite the separated analytes of a given sample in channels 72 as they pass by the laser position. Fluorescence emission from sample analytes is detected by multichannel fluorescence detector 84 and presented in any conventional manner to give the results of the specific separation.

Laser 83 may be positioned at the portion of the microchip where channels 72 are very closely spaced, as shown in Fig. 6. Alternatively, if analyte separation is carried out in capillaries 73 instead of in the channels 72 of the microchip, it may be preferable to position the laser just beyond connecting structures 74 so that detection can be carried out before the bands of separated analytes can disperse. Upon the completion of a specific analysis, the electrical circuit can be disconnected, capillaries 73 can be removed from the sample wells, and pump 80 can be used to pump washing fluid through the

matrix in channels 72 or to replace the matrix completely, in preparation for analysis of additional samples.

While the present invention has been described in conjunction with a preferred embodiment, one of ordinary skill, after reading the foregoing specification, will be able to effect various changes, substitutions of equivalents, and other alterations to the compositions and methods set forth herein. It is therefore intended that the protection granted by Letters Patent hereon be limited only by the definitions contained in the appended claims and equivalents thereof.

CLAIMS

What is claimed is:

1. A hybrid microfabricated substrate capillary array assembly comprising:

one or more capillaries each having a first cross-sectional shape;

a microfabricated substrate having one or more channels formed therein for conducting a fluid in said substrate, said one or more channels each having a second cross-sectional shape; and

connecting structure formed in said substrate connecting said one or more capillaries to said one or more channels in said substrate so as to enable fluid communication between said capillaries and said channels in said substrate.

2. The hybrid assembly of claim 1 wherein said microfabricated substrate has a plurality of channels, said channels are substantially parallel to one another, and said channels lie substantially in the same plane.

3. The hybrid assembly of claim 1 wherein both said first and said second cross-sectional shapes are circular.

4. The hybrid assembly of claim 1 wherein said one or more channels are configured for manipulation of fluid suspended molecular species conducted through said channels.

5. The hybrid assembly of claim 4 wherein said manipulation step comprises separation of said fluid suspended molecular species.

6. The hybrid assembly of claim 4 wherein said manipulation step comprises reaction of said fluid suspended molecular species.

7. The hybrid assembly of claim 1 wherein said plurality of capillaries are configured for manipulation of fluid suspended molecular species conducted through said capillaries.

8. The hybrid assembly of claim 7 wherein said manipulation step comprises separation of said fluid suspended molecular species.

9. The hybrid assembly of claim 7 wherein said manipulation step comprises reaction of said fluid suspended molecular species.

10. The hybrid assembly of claim 1 wherein said microfabricated substrate is a light transmissive material.

11. The hybrid assembly of claim 1, said microfabricated substrate having a plurality of channels, said channels at a first end of each having separate ports in one or more surfaces

of said substrate, said channels at a second end of each terminating in a common port in a surface of said substrate.

12. The hybrid assembly of claim 11 wherein said common port is configured for enabling fluid washing of said channels.

13. The hybrid assembly of claim 11 wherein said common port is configured for enabling replacement of fluid media in said channels.

14. A microfabricated substrate having a plurality of channels formed therein for conducting a fluid in said substrate, said channels at a first end of each having separate ports in one or more surfaces of said substrate, said channels at a second end of each terminating in a common port in a surface of said substrate.

15. The microfabricated substrate of claim 14 wherein said common port is configured for enabling fluid washing of said channels.

16. The microfabricated substrate of claim 14 wherein said common port is configured for enabling replacement of fluid media in said channels.

17. The microfabricated substrate of claim 14 wherein said substrate is a light transmissive material.

18. A multichannel detection assembly comprising:

a light transmissive microfabricated substrate having a plurality of channels formed therein for conducting a fluid in said substrate, wherein, in a portion of said substrate, said channels are positioned substantially parallel to each other and define a plane in said substrate;

a light source having an output directed through said substrate portion at an angle to said plane, wherein said angle is less than the critical reflective angle of said substrate, said light source output travelling through said plurality of channels; and

a detector positioned adjacent to said substrate portion to receive light transmitted through said channels in said substrate.

19. The detection assembly of claim 18 wherein, in said microfabricated substrate, said channels each have a first end and a second end and said channels are more closely spaced at said second end than said first end.

20. The detection assembly of claim 19 wherein said light source is positioned so that said light source output travels through

said plurality of channels closer to said second end of said channels than to said first end of said channels.

21. The detection assembly of claim 18 wherein said light source is a laser.

22. The detection assembly of claim 18 wherein said light source output is ultraviolet light.

23. The detection assembly of claim 18 wherein said detector is a fluorescence detector.

24. The detection assembly of claim 18 wherein said detector is an absorbance detector.

25. The detection assembly of claim 18 further comprising a plurality of capillaries connected to said plurality of channels in said substrate.

26. A microfabricated hybrid capillary array and multichannel detection assembly comprising:

a plurality of capillaries each having a circular cross-sectional shape;

a light transmissive microfabricated substrate having a plurality of channels formed therein for conducting a fluid in said substrate, said channels having a circular cross-sectional

shape, wherein, in a portion of said substrate, said channels are positioned substantially parallel to each other and define a plane in said substrate;

connecting structure formed in said substrate connecting said capillaries to said channels so as to enable fluid communication between said capillaries and said channels;

a light source having an output directed through said substrate at an angle to said plane, wherein said angle is less than the critical reflective angle of said substrate, said light source output travelling through said plurality of channels; and

a detector positioned adjacent to said substrate portion to receive light transmitted through said channels in said substrate.

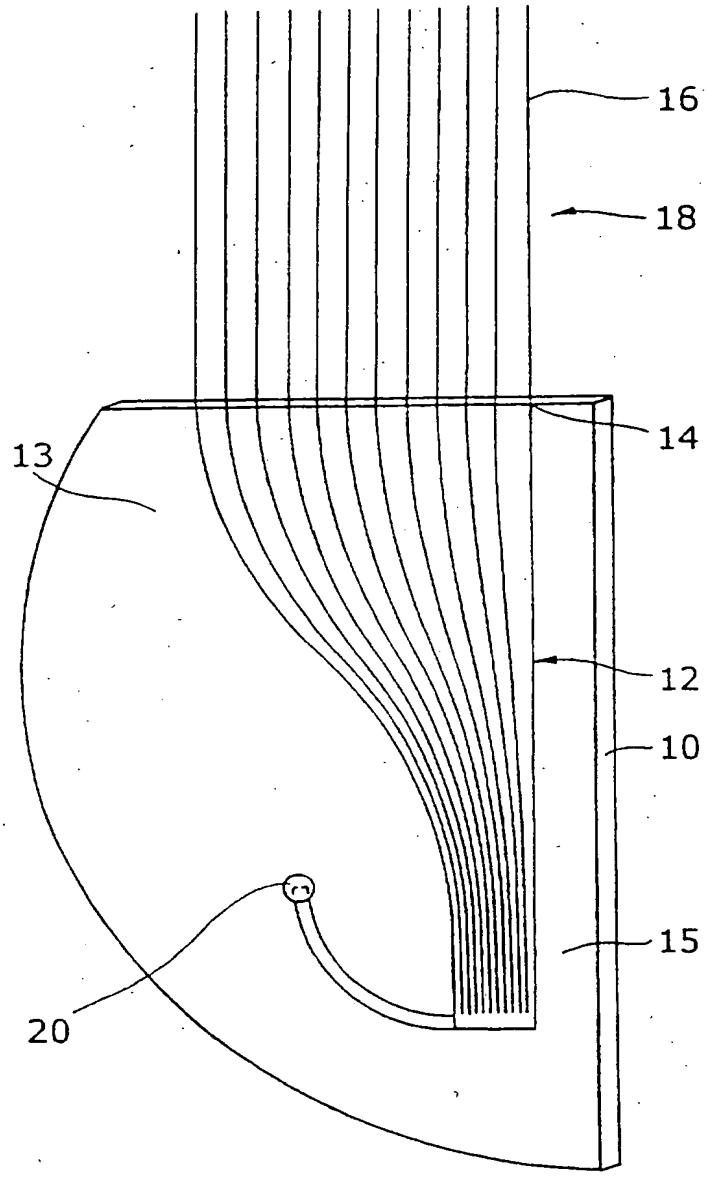

FIG. 1

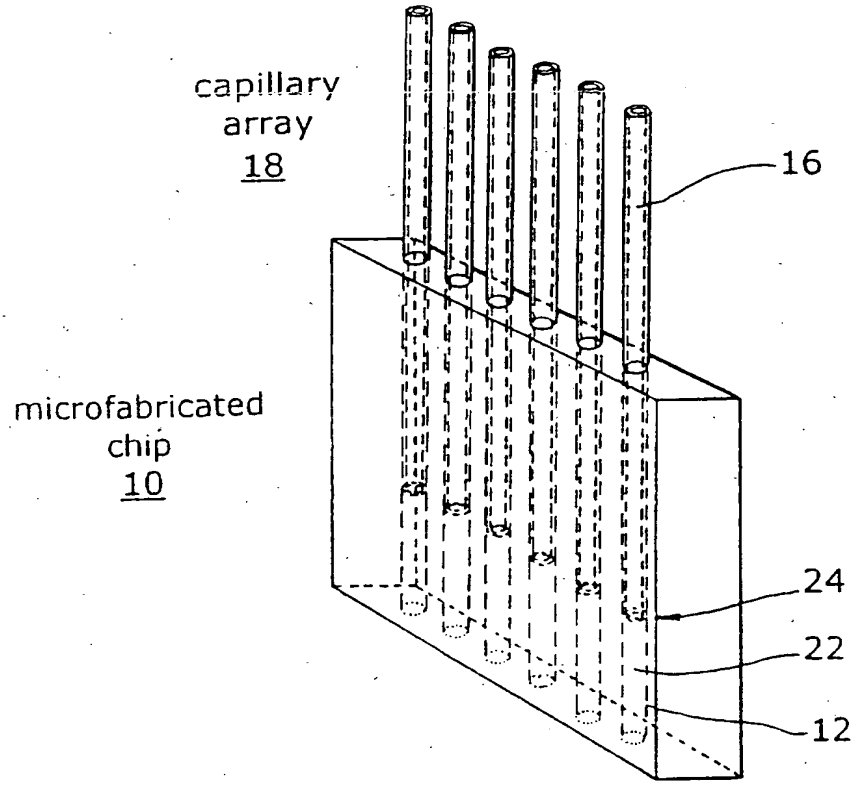

FIG. 2A

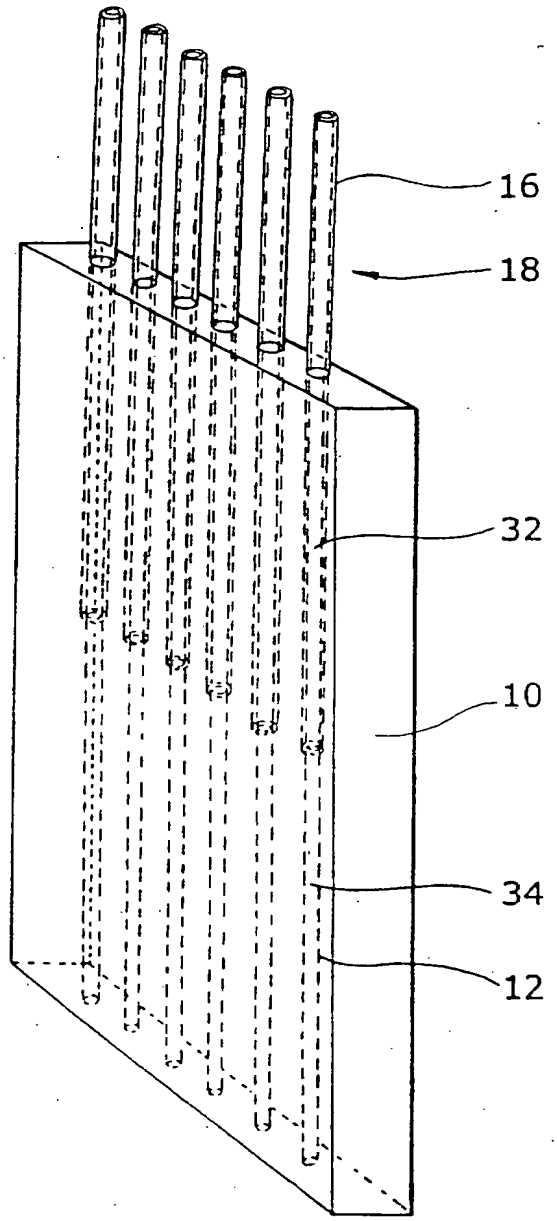

FIG. 2B

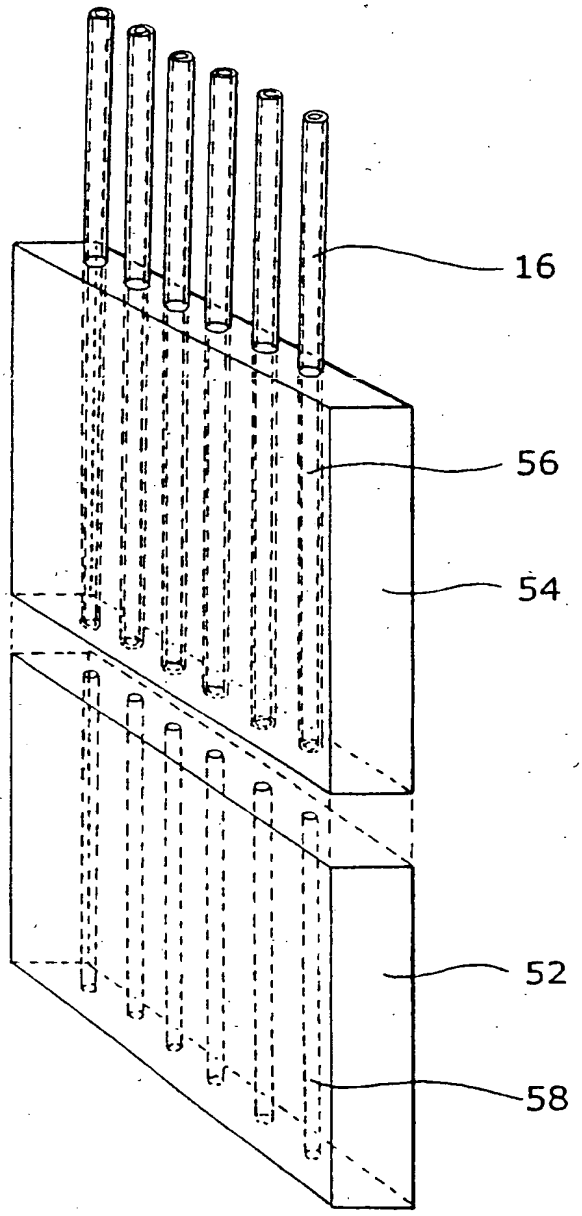

FIG. 2C

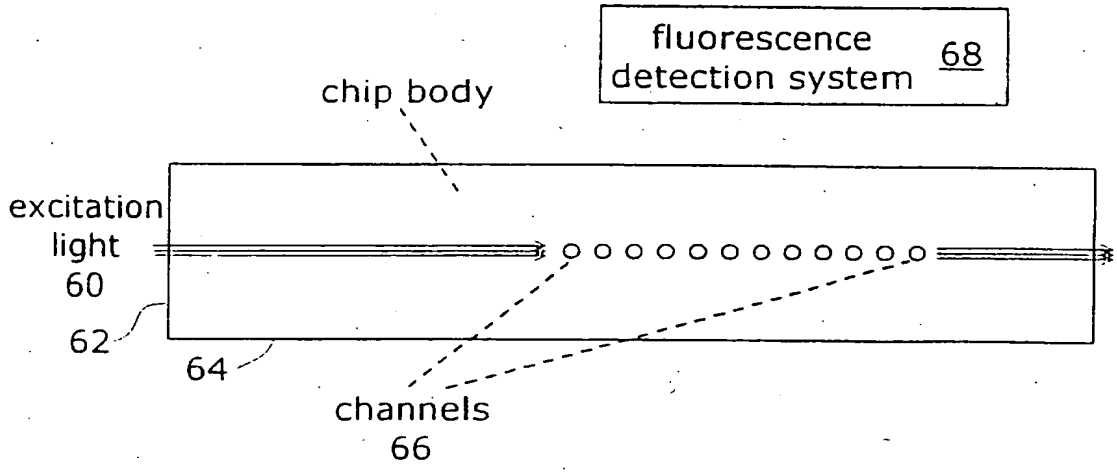

FIG. 3A

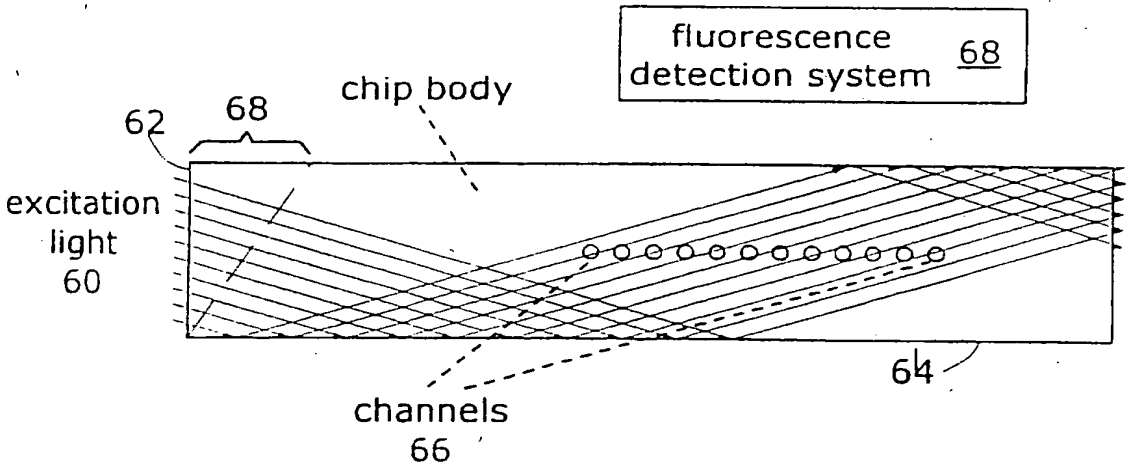

FIG. 3B

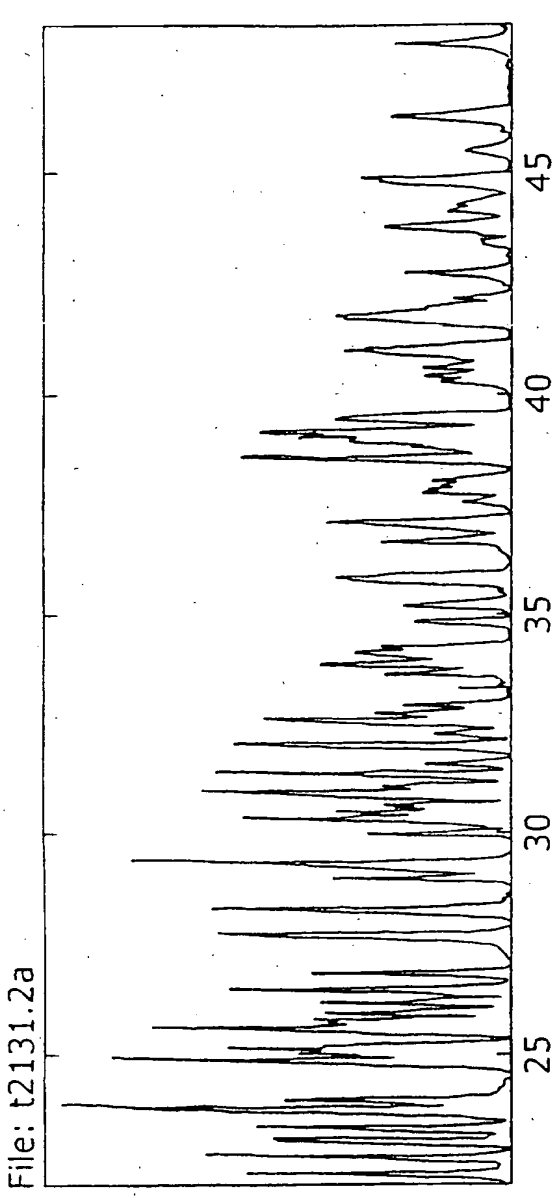

FIG. 4A

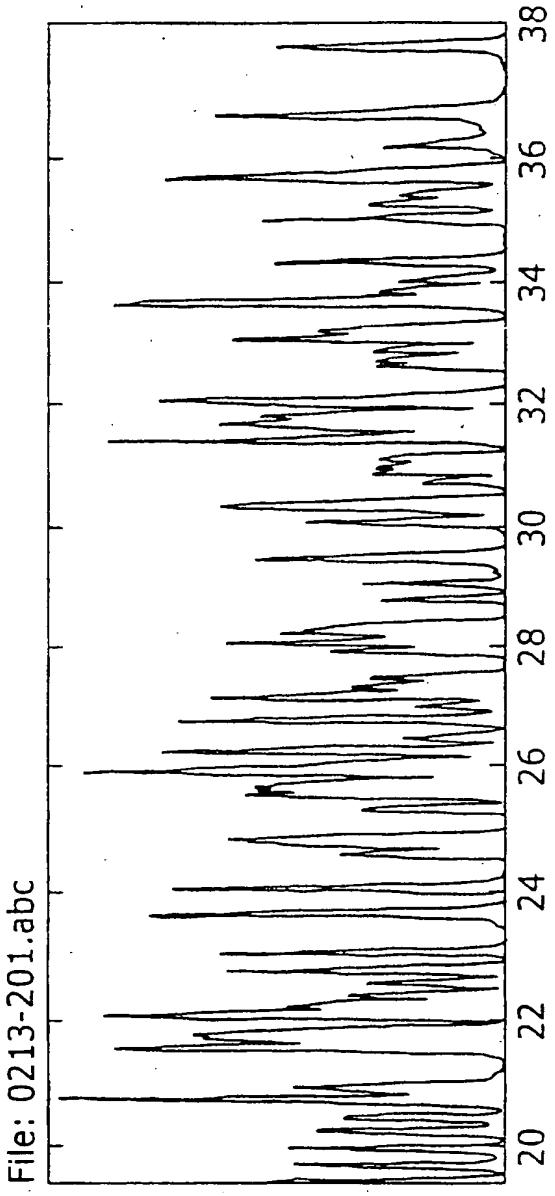

FIG. 4B

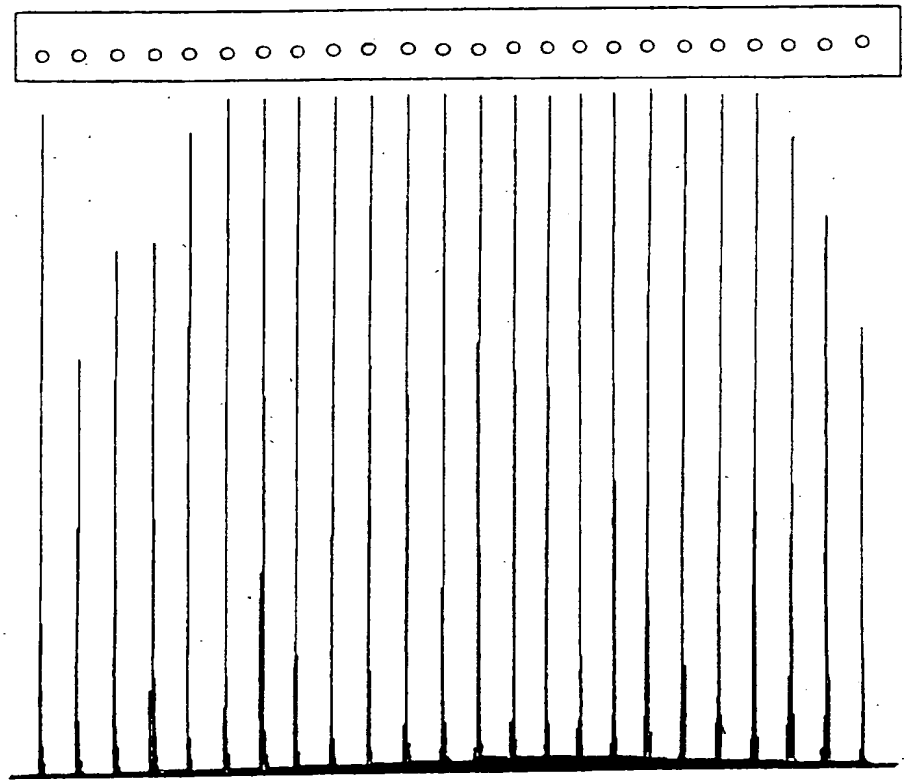

FIG. 5

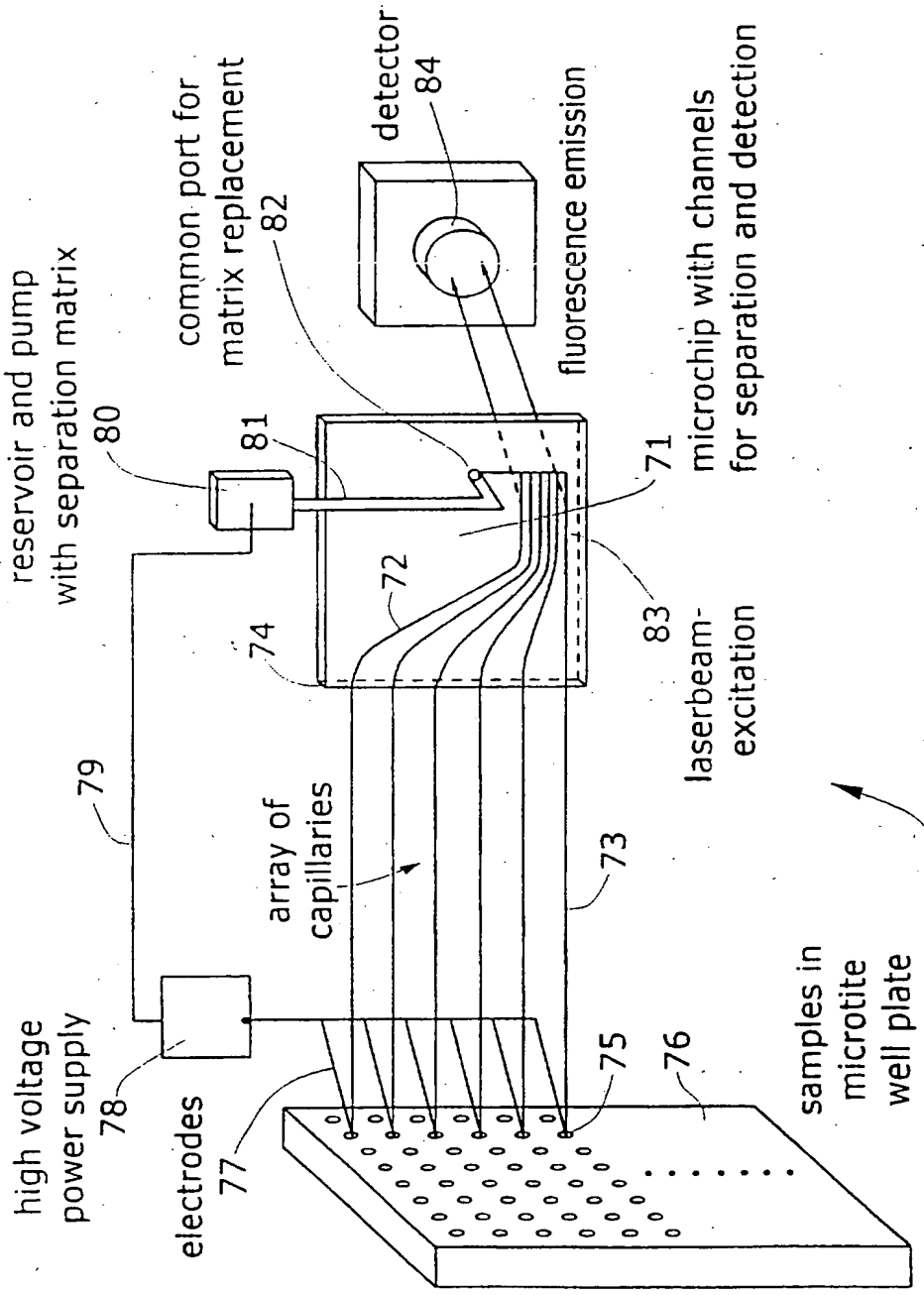

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/15461

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C25B 9/00, G01N 27/26, 27/447

US CL :356/318, 344; 204/299 R, 182.8, 603, 452

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 356/318, 344; 204/299 R, 182.8, 603, 452

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,366,608 A (Kambara) 22 November 1994 (22/11/94), see entire document.	1-3,10,11, 18-26
Y	US 4,908,112 A (Pace) 13 March 1990 (13/03/90), see entire document, especially column 3-4, lines 27-62.	4-9,14,16, 17
Y,E	US 5,674,743 A (Ulmer)07 October 1997 (07/10/97), see entire document.	12,13,15

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 20 OCTOBER 1997	Date of mailing of the international search report 12 NOV 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MICHAEL P. STAFIRA Telephone No. (703)308-0956

Form PCT/ISA/210 (second sheet)(July 1992)*